

Appl. No. : 09/738,046  
Filed : December 15, 2000

Two variations of the method were tested. In one, lipid formulation was first resuspended in hydration buffer to form liposomes and then antibody-oligonucleotide conjugate was added to the liposome formulation. This approach leads to the formation of lipoplexes. In another variation, antibody-oligonucleotide conjugate was directly added to the dried film of BioPORTER reagent. This approach leads to encapsulation of the protein-oligonucleotide conjugates as well as lipoplex formation. Either approach was found to be successful in the intracellular delivery of antibody-oligonucleotide conjugates.

#### REMARKS

The specification has been amended in order to conform with the sequence listing that is submitted herewith.

#### CONCLUSION

The specific changes to the specification are shown on a separate set of pages attached hereto and entitled **VERSION WITH MARKINGS TO SHOW CHANGES MADE**, which follows the signature page of this Amendment. On this set of pages, the insertions are bolded and underlined.

Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 11-1410.

Respectfully submitted,

KNOBBE, MARTENS, OLSON & BEAR, LLP

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By: 

Ned A. Israelson

Registration No. 29,655  
Attorney of Record  
620 Newport Center Drive  
Sixteenth Floor  
Newport Beach, CA 92660  
(619) 235-8550

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**VERSION WITH MARKINGS TO SHOW CHANGES MADE**

**The paragraph beginning on page 14, line 17, has been amended as follows:**

In one embodiment, peptide nucleic acid (PNA) clamping technology is used to attach proteins to plasmid DNA. PNA clamps may be used to attach various ligands, including proteins and peptides, onto DNA. This technology is called "PNA dependent gene chemistry" (PDGC) and is described by Zelphati, et al., *BioTechniques* 28: 304-310 (2000), in PCT WO98/19503, and in co-pending U.S. Patent Application Serial No. 09/224,818, the entire contents of which are incorporated herein by reference. PNA is a polynucleotide analog that has the deoxyribose-phosphate backbone of DNA replaced by a peptide backbone (Fig. 3; SEQ ID NO. 1). The PNA clamp hybridizes with its complementary binding site on a plasmid to form a highly stable PNA-DNA-PNA triplex clamp.

**The paragraph beginning on page 14, line 26, has been amended as follows:**

A plasmid, pGeneGrip<sup>TM</sup>, is available from Gene Therapy Systems, Inc. (San Diego, CA) that contains PNA binding sites as shown in Fig. 3 (SEQ ID NO. 2). Several different labeled PNA clamps can be used, including PNA labeled with biotin, reactive chemical groups such as maleimide, and fluorescent labels such as rhodamine and fluorescein. An 80 base pair polypurine -AG- repeat sequence (pGeneGrip site) was cloned after the terminator of a cytomegalovirus (CMV) immediate early gene promoter-based plasmid. This region of the plasmid was selected for insertion of the binding site because it is not involved in transcription and PNA binding to this region does not affect expression (Zelphati et al., *Hum. Gene Ther.* 10: 15-24, 1999). A complementary PNA clamp was synthesized consisting of an 8 base -CT-repeat, a 3 unit flexible linker (8-amino-3,6-dioxaoctanoic acid), and an 8 base -JT-repeat, where J is pseudouracil, an analog of C, which encourages formation of the Hoogsteen triplex hybrid (Zelphati et al., 1999, *supra*; Egholm et al., *Nucl. Acids Res.* 23: 217-222, 1995). The -CT- stretch hybridizes to the -AG- repeat on the plasmid in an anti-parallel Watson-Crick manner, and the -JT- stretch binds in the major groove of the PNA-DNA hybrid via Hoogsteen interactions to form the PNA-DNA-PNA triplex clamp (Egholm et al., *supra*). The non-target DNA strand is displaced, forming the non-hybridized "D-loop" (Bukanov et al., *Proc. Natl. Acad. Sci. U.S.A.* 95: 5516-5520, 1998; Cherny et al., *Proc. Natl. Acad. Sci. U.S.A.* 90: 1667-1670, 1993).

**The paragraph beginning on page 27, line 11, has been amended as follows:**

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An oligonucleotide obtained from a commercial supplier (GenBase, Inc.) containing a 5' terminal NH<sub>2</sub> group and a 3' terminal Rhodamine moiety (5'-NH<sub>2</sub>-TGACTGTGAAACGTTCGAGATGA-Rhodamine-3'; SEQ ID NO. 3) was conjugated to goat IgG (Sigma) and was introduced into cells using a conventional cationic lipid transfection reagent. Two variations of the method were tested. In one, lipid formulation was first resuspended in hydration buffer to form liposomes and then antibody-oligonucleotide conjugate was added to the liposome formulation. This approach leads to the formation of lipoplexes. In another variation, antibody-oligonucleotide conjugate was directly added to the dried film of BioPORTER reagent. This approach leads to encapsulation of the protein-oligonucleotide conjugates as well as lipoplex formation. Either approach was found to be successful in the intracellular delivery of antibody-oligonucleotide conjugates.